**ACT HEALTH PROTECTION SERVICE**

# MICROBIOLOGICAL QUALITY OF SEED SPROUTS OCTOBER – DECEMBER 2014

Prepared by Natasha Waters, Radomir Krsteski and Simon Rockliff

## BACKGROUND/OBJECTIVE

Seed sprouts contaminated by pathogenic micro-organisms present an unacceptable health risk to consumers. Sprouts have been implicated in a number of outbreaks both in Australia and Overseas.

The consumption of contaminated seed sprouts presents a risk because they are commonly consumed raw in salads, sandwiches and garnishes or in flash cooked foods such as stir-fries. They can be sold directly to the consumer in packaged forms at greengrocers, markets, supermarkets and Asian grocers. It has also been shown that washing sprouts in water does not remove bacteria and only reduces numbers of *Escherichia coli* (*E.coli)* and *Salmonella* by no more than 1-log (Taormina et al, 1999).

Seed sprouts are defined as:
*"Seed sprouts are sprouted seeds or sprouted beans for human consumption that include all or part of the seed (FSANZ, 2011)."*

Snow pea sprouts are commonly referred to as "sprouts" but differ in the manufacturing in that they are grown in a growth medium (enriched soil) and cut on the stem away from the roots and therefore do not include the seed (FSANZ 2011). The scope of this survey included snow pea sprouts as there are still risks associated with consuming this product as raw in a salad or garnish. Further, it is unlikely that these types of products will be covered by another survey. These results are collated separately.

Seed sprouts pose a particular risk as the seed itself can carry pathogens with the sprouting process supporting microbial growth. The seeds themselves have been identified as the likely pathogenic source in many reported outbreaks of food-borne illness linked to seed sprouts (FSANZ, 2010). Other possible sources of contamination during the germination and sprouting phase are; water, pests, and the growing medium. Harvesting can also lead to contamination through rinse water, equipment and the workers themselves (FSANZ, 2010).

In April-June of 2001 the Australian Capital Territory (ACT) conducted a survey of seed sprouts. It found that the quality of seed sprouts were generally good but occasional batches tested contained pathogens (Millard, 2001).

Between 1988 and 2008 there have been over forty reported outbreaks worldwide attributed to the consumption of contaminated seed sprouts (FSANZ, 2011). During 2005 to 2006 there have been 140 reported cases in Australia of food borne salmonellosis associated with the consumption of alfalfa sprouts (FSANZ, 2011).

In 2011 the largest recorded international outbreak of Shiga toxin producing *E. coli* (STEC) occurred in Germany. It comprised of a total of 3816 cases including fifty four deaths. Up to fifteen other countries also recorded cases from people who had

travelled to northern Germany during the outbreak. This outbreak was attributed to the consumption of contaminated fenugreek sprouts (OZfoodNet, 2011).

In 2012 a survey conducted by the Victorian Department of Health assessed the microbiological status of 298 seed sprout products sold and used by small businesses and supermarkets across Victoria. It was found that overall the microbiological quality of unpackaged samples were not as good as packaged products. *Salmonella* were not detected however *Listeria monocytogenes* (*L. monocytogenes*) and *E. coli* were detected in some samples.

Numerous surveys have also been conducted by the New South Wales food authority in 2005, 2006 and 2008 with some samples being positive for *L. monocytogenes* and *E. coli*.

This survey was undertaken as a survey has not been conducted in the ACT since 2001 and these products may still present a risk to Australian consumers.

## OBJECTIVE

The main objectives of the Seed sprout survey were:

- To examine whether these products are a potential source of contaminated by microbiological organisms such as *B cereus*, *E coli*, *Salmonella* spp and *L. monocytogenes and* collate seed sprouts as separate from snow pea sprouts.

- To assess the microbiological quality of the Seed Sprouts and snow pea sprouts based on the Australia and New Zealand Food Standard (FSANZ) Guidelines for the microbiological examination of Ready-to-Eat (RTE) foods (FSANZ RTE Guidelines).

- To identify any link between sample source and microbiological quality by comparing the results of the seed sprout samples collected from different types of outlets in ACT such as supermarkets, Asian shops and farmers markets.

## STANDARDS

The FSANZ RTE Guidelines identify four categories of microbiological quality ranging from satisfactory to potentially hazardous. Table 1 details the recommended guideline value. Table 1 not only reflects both the high level of microbiological quality that is achievable for RTE foods in Australia and New Zealand but also indicates the level of contamination that is considered to be a significant risk to the public health.

**Table 1**

| Test | Microbiological Quality (colony forming units per gram (cfu/g)) | | | |
|---|---|---|---|---|
| | **Satisfactory** | **Marginal** | **Unsatisfactory** | **Potentially Hazardous** |
| **Indicators** | | | | |
| *E. coli* | <3 | 3-100 | >100 | ** |
| *B. cereus* | $<10^2$ | $10^2$-$10^3$ | $10^3$-$10^4$ | Greater than or equal to $10^4$ |
| **Pathogens** | | | | |
| *Salmonella spp.* | not detected in 25g | | | detected |
| *L. monocytogenes* | not detected in 25g | detected but $<10^{2\ \#}$ | | $\geq 10^{2\ \#\#}$ |

**NOTE:**
** Pathogenic strains of *E. coli* should be absent.
# Foods with a long shelf life stored under refrigeration should have no *L. monocytogenes* detected in 25g.
## The detection of *L. monocytogenes* in ready-to-eat-foods prepared specifically for "at risk" population groups (the elderly, immuno-compromised and infants) should also be considered as potentially hazardous.

## SURVEY

This survey was conducted between October and December 2014. During this period fifty seven samples from eleven ACT retail outlets were collected randomly by Health Protection Service (HPS) Public Health Officers (PHO) and processed by ACT Government Analytical Laboratory. This consisted of twelve snow pea sprouts samples and forty five seed sprout samples.

All fifty seven samples were tested for the hygiene indicator *E.coli* and food pathogens *L. monocytogenes, Salmonella* and *B. cereus*. Multiple samples of different sprout types were collected from each food outlet; this may have also included duplicates of the same type of sprout. If multiple samples of the same sprout were collected then PHO attempted to sample different batch/use by dates.

Where the HPS identifies non compliance issues in food businesses, corrective actions are addressed through a graduated and proportionate response. Unsatisfactory results, excluding those for SPC are re-sampled. Marginal results may be re-sampled; this is dependent on resources as these foods are still considered compliant. Unsatisfactory SPC results are not re-sampled unless pathogens are also isolated. However if the leftover food items are not available for re-analysis, other food items from the premises are collected for testing.

## MICROBIOLOGICAL METHOD OF ANALYSIS

Samples were tested for the presence of:

- *Salmonella* based on AS 5013.10 – 2009 (modified)
- *E. coli* based on ISO: 16649.2 -2001

- *L. monocytogenes* based on AS 5013.24.1-2009 (modified)
- *B. cereus* based on AS 5013.2-2007**.**

The sample preparation for *E. coli and B. cereus* consisted of:

- 25g of sample being homogenised with 225mL of 0.1% peptone diluents
- subsequent serial dilutions were prepared for use in enumeration.

***E. coli* enumeration:** Pour plates of TBX agar using 1ml of $10^{-1}$ dilution were prepared in triplicate and incubated at 37ºC/4 h followed by 44ºC/20 h. *E. coli* colonies appear blue/green after incubation.

***B. Cereus* enumeration**: Spread plates of Mannitol, Egg-Yolk, Polymixin Agar (MYP) agar were prepared in duplicate at $10^{-2}$ and a single plate at $10^{-4}$ and incubated for 24hrs at 30˚C. Plates were then checked for growth of any suspect colonies. Plates were then re-incubated for a total incubation time of 48hrs at 30˚C. No confirmation testing was carried out

***Salmonella* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL buffered peptone water with novobiocin and incubated at 42°C/24h. Aliquots were then transferred into Brain Heart Infusion broth (BHI) and incubated for 3 hrs. DNA was extracted from 200uL of enriched BHI. This was screened for the presence of salmonella using a BAX cyber green Polymerase Chain Reaction (PCR). No confirmation testings were required to be performed as the samples were not screened positive in PCR.

***L. monocytogenes* detection:** 25g of sample was weighed out aseptically and homogenised with 225mL Half Fraser broth (selective enrichment) and incubated at 30°C/24h. Aliquots were then transferred into a single tube of Fraser broth and MOPS BLEB broth which were incubated at 37°C/48h and 37°C/24h respectively. DNA was extracted from 200uL of enriched MOPS BLEB broth. This was screened for the presence of *L. monocytogenes* using a BAX cyber green PCR. Confirmation tests were not performed as the samples were not screened positive.

## RESULTS / DISCUSSION

### *E. coli*

Altogether fifty seven survey samples were tested for the hygiene indicator *E. coli*. Forty five of these samples were seed sprouts and twelve were snow pea sprouts. There was no *E.coli* detected in any of the snow pea sprouts samples (100%). Forty two seed sprouts (93.3%) samples were found to be satisfactory as *E. coli* was not detected (<3 cfu/g).

Three seed sprout samples (6.7%) were found to be marginal as *E coli* were detected within the range of marginal level (3-100 cfu/g). According to the food FSANZ guidelines, resample is not required if the results are reported marginal. Therefore, re-sampling was not conducted in these cases.

The presence of generic *E. coli* in ready to eat foods is undesirable. Its presence in food indicates the poor sanitation and hygienic conditions which have lead to contamination. The detection of *E. coli* in RTE food does not reflect that the food is unsafe rather it is an indication of potential problems involving sanitation and poor food handling.

***Salmonella***
All of the fifty seven samples were tested for *Salmonella.* No *Salmonella* was detected in any sample. All fifty seven samples (100%) met the satisfactory criteria for this organism.

RTE foods should be free of *Salmonella* as consumption of foods containing this pathogen may result in foodborne illness.

***L. monocytogenes***
All of the samples were analysed for *L. monocytogenes*. No *L. monocytogenes* was detected in any sample. All fifty seven samples (100%) met the satisfactory criteria for this organism.

The detection of *L. monocytogenes* in such foods indicates the food was inadequately prepared or the food was contaminated post preparation. The detection of higher levels ($>10^2$ cfu/g) of *L. monocytogenes* in RTE foods indicates a failure of food handling controls and is also considered a public health risk.

All RTE foods are tested for the presence of *L. monocytogenes* in 25g. If *L. monocytogenes* is detected PHO will inspect the premises and collect a resample of the food item if available. This re-sample will be tested in a semi-quantitative manner to measure the level of *L. monocytogenes* in the food.

## CONCLUSION

The microbiological quality of the seed sprouts and snow pea sprouts surveyed in the ACT was found to be good. No harmful pathogens such as *Salmonella* and *L. monocytogenes* were detected in survey samples. However the survey found marginal levels of generic *E. coli* in three samples of seed sprouts (6.38%). Raw results of the analysis are attached at Appendix A.

Although the survey set out to sample sprouts from ACT supermarkets, farmer's markets, Fyshwick/Belconnen markets and Asian grocers, only one of the sampling locations was not a supermarket. Future surveys should take into account other sources of samples.

Surveys run by NSWFA have also included sprouts sampled from takeaways, restaurants, sandwich bars; health food shops and any other food business which sold packaged or unpackaged seed sprout products. They found the microbiological quality of the unpackaged products overall was not as good as packaged products samples.

## BIBLIOGRAPHY


1. FDA National Advisory Committee on Microbiological Criteria for Food 1999, Microbiological Safety Evaluations and Recommendations on Sprouted Seeds.
2. Food Standards Australia New Zealand (FSANZ), 2001, Guidelines for the microbiological examination of ready-to-eat foods.
3. Food Standards Australia New Zealand (FSANZ) 2011, Proposal P 1004 – Primary Production & Processing Standard for Seed Sprouts- Executive Summary.
4. Food Standards Australia New Zealand (FSANZ) 2010, Proposal P 1004 – Primary Production & Processing Standard for Seed Sprouts- Technical Paper.
5. Goldsmith P 2012, Microbiological surveillance of seed sprouts products across Victoria- Final report December 2012.
6. Millard G & Rockliff S 2001, Microbiological Quality of Seed Sprouts April-June 2001.
7. Taormina PJ et al 1999, Infections Associated with Eating Seed Sprouts: An International Concern.
8. **The OzFoodNet Working Group 2011, Monitoring the incidence and causes of diseases potentially transmitted by food in Australia: Annual report of the OzFoodNet network.**
9. **The OZFoodNet Working Group 2006, OZFoodNet: Quarterly report, 1 April to 30 June 2006, Communicable Disease Intelligence.**
10. http://cdn.morguefile.com/imageData/public/files/j/JessicaGale/04/h/14290539372hylz.jpg

## Appendix A

| Type of Sprout | *B. cereus* | *E. coli* | *L. monocytogenes* | *Salmonella* spp |
|---|---|---|---|---|
| | cfu/g | cfu/g | Presence/Absence in 25g | Presence/Absence in 25g |
| **Mung bean** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Bean** | <50 | <3 | Absent | Absent |
| **Mixed** | <50 | <3 | Absent | Absent |
| **Mixed** | <50 | <3 | Absent | Absent |
| **Mixed** | <50 | <3 | Absent | Absent |
| **Mung bean** | <50 | <3 | Absent | Absent |
| **Mixed Spouts (mung bean, blue peas, chickpeas, lentils)** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | 7 | Absent | Absent |
| **Mung bean** | <50 | 7 | Absent | Absent |
| **Mung bean** | <50 | 3 | Absent | Absent |
| **Soya** | <50 | <3 | Absent | Absent |
| **Soya** | <50 | <3 | Absent | Absent |
| **Soya** | <50 | <3 | Absent | Absent |
| **Mixed sprouts (peas and lentil)** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Mung bean** | <50 | <3 | Absent | Absent |
| **Mung bean** | <50 | <3 | Absent | Absent |
| **Mixed sprouts** | <50 | <3 | Absent | Absent |
| **Mixed sprouts** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Mixed Sprouts** | <50 | <3 | Absent | Absent |
| **Mixed Sprouts** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Mung bean** | <50 | <3 | Absent | Absent |
| **Bean** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Mung bean** | <50 | <3 | Absent | Absent |
| **Alfalfa and radish** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Mixed sprouts** | <50 | <3 | Absent | Absent |

| | | | | |
|---|---|---|---|---|
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Alfalfa and Onion** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Mixed sprouts** | <50 | <3 | Absent | Absent |
| **Alfalfa and Broccoli** | <50 | <3 | Absent | Absent |
| **Alfalfa and Broccoli** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Alfalfa** | <50 | <3 | Absent | Absent |
| **Alfalfa, onion and garlic chive** | <50 | <3 | Absent | Absent |
| **Alfalfa, onion and garlic chive** | <50 | <3 | Absent | Absent |
| **Mixed sprouts** | <50 | <3 | Absent | Absent |
| **Snow pea** | <50 | <3 | Absent | Absent |